Davis
10/030390


```
     FILE 'REGISTRY' ENTERED AT 13:47:38 ON 09 OCT 2003
                E TFF1/CN 5
L5              1 S E4
                E TREFOIL PEPTIDE/CN 5
L6              4 S E4-E7
L7              5 S L5 OR L6

     FILE 'HCAPLUS' ENTERED AT 13:57:28 ON 09 OCT 2003
L5              1 SEA FILE=REGISTRY ABB=ON  PLU=ON  "TFF1 (HUMAN)"/CN
L6              4 SEA FILE=REGISTRY ABB=ON  PLU=ON  ("TREFOIL PROTEIN
                  (HUMAN CLONE HAGGT12)"/CN OR "TREFOIL PROTEIN (HUMAN
                  CLONE HNECF58)"/CN OR "TREFOIL PROTEIN (HUMAN CLONE
                  HSLAW55)"/CN OR "TREFOIL PROTEIN (HUMAN CLONE HYACJ67)"/C
                  N)
L7              5 SEA FILE=REGISTRY ABB=ON  PLU=ON  L5 OR L6
L8            302 SEA FILE=HCAPLUS ABB=ON  PLU=ON  ((GASTROINTESTIN? OR
                  GASTR? INTESTIN? OR GASTRIC OR INTESTIN?)(S)(DISEAS? OR
                  DISORDER) OR CROHN? OR REGIONALIS OR COLITIS OR ULCER?
                  OR ENTERITIS OR ILEITIS OR ILEOCOLITIS OR RECTOCOLITIS
                  OR PROCTOCOLITIS) AND (LACTOCOCC? OR LACTOBACILL? OR
                  LACTIS)
L9              1 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L8 AND (L7 OR (OESTROGEN
                   OR ESTROGEN)(1W)PEPTIDE OR TREFOIL OR TFF# OR PS2 OR
                  PS(1W)2)
```

- key terms

L9 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2001:31647 HCAPLUS
DOCUMENT NUMBER: 134:95499
TITLE: Transformed **Lactococcus** or **Lactobacillus** containing recombinant plasmid vectors encoding **trefoil** peptide **TFF1** used for treatment of **gastric** and/or **intestinal disorders**
INVENTOR(S): Hans, Wolfgang Christian; Steidler, Lothar; Remaut, Erik Rene
PATENT ASSIGNEE(S): Vlaams Interuniversitair Instituut voor Biotechnologie, Belg.
SOURCE: PCT Int. Appl., 44 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001002570	A1	20010111	WO 2000-EP6343	20000705
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1194554	A1	20020410	EP 2000-954434	20000705

Searcher : Shears 308-4994

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

JP 2003504025 T2 20030204 JP 2001-508342 20000705

PRIORITY APPLN. INFO.: EP 1999-870143 A 19990705
WO 2000-EP6343 W 20000705

AB The invention provides recombinant plasmid vectors containing DNA sequences encoding the mature mouse **trefoil** peptide **TFF1** (originally named **pS2**), under the control of a suitable promoter. The invention also provides the use of said recombinant plasmid vector in transforming **Lactococcus** or **Lactobacillus** species, and methods used for producing recombinant **TFF1** in said bacteria. The invention further provides a pharmaceutical composition comprising the recombinant **Lactococcus** or **Lactobacillus**. Still further, the invention provides for the use of recombinant **Lactococcus** or **Lactobacillus**: (1) in treatment of **gastric** and/or **intestinal diseases** or **disorders**, such as acute **colitis**, acute flare ups of **Crohn's disease** and **ulcerative colitis**, and (2) in treatment or inhibition of lesions caused by such **diseases**. Finally, the invention provides the DNA sequence of the recombinant plasmid vectors, pL2mTFF1v1, pT1mTFF1 and pPICmTFF1, which encode part or all of the mouse mature **TFF1** peptide. In the example section, the invention showed that mice, suffering from acute **colitis**, showed a significant reduction of intestinal inflammation when inoculated with recombinant L. **lactis** containing plasmid pT1mTFF1. The invention also presented the construction of plasmid pPICmTFF1, which was used to recombinantly produce **TFF1** using Pichia pastoris.

REFERENCE COUNT: 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

(FILE 'MEDLINE' ENTERED AT 14:01:09 ON 09 OCT 2003)

L12	17240	SEA FILE=MEDLINE ABB=ON	PLU=ON	"CROHN DISEASE"/CT	
L13	6677	SEA FILE=MEDLINE ABB=ON	PLU=ON	COLITIS/CT	
L14	235	SEA FILE=MEDLINE ABB=ON	PLU=ON	LACTOCOCCUS/CT	
L15	6712	SEA FILE=MEDLINE ABB=ON	PLU=ON	LACTOBACILLUS/CT	
L16	40	SEA FILE=MEDLINE ABB=ON	PLU=ON	(L12 OR L13) AND (L14 OR L15)	
L17	20	SEA FILE=MEDLINE ABB=ON	PLU=ON	L16 AND (THERAPY OR THERAPEUTIC USE)/CT	

L17 ANSWER 1 OF 20 MEDLINE on STN
AN 2003294904 MEDLINE
TI Double blind, placebo controlled trial of two probiotic strains in interleukin 10 knockout mice and mechanistic link with cytokine balance.
AU McCarthy J; O'Mahony L; O'Callaghan L; Sheil B; Vaughan E E; Fitzsimons N; Fitzgibbon J; O'Sullivan G C; Kiely B; Collins J K; Shanahan F
SO GUT, (2003 Jul) 52 (7) 975-80.
Journal code: 2985108R. ISSN: 0017-5749.
AB BACKGROUND: Prophylactic efficacy against colitis following lactobacillus consumption in interleukin 10 (IL-10) knockout (KO) mice has been reported. Whether this applies equally to other probiotic strains is unknown, and the mechanism is unclear. AIMS: (1) To compare the effect of feeding Lactobacillus salivarius

subspecies salivarius 433118 and Bifidobacterium infantis 35624 against placebo on enterocolitis, the intestinal microflora, and (2) to compare the systemic immunological response to in vitro microbial challenge in probiotic fed and control IL-10 KO mice. METHODS: Three groups of 10 IL-10 KO mice were fed fermented milk products containing Lb salivarius 433118 at 10(9) CFU/ml, B infantis 35624 at 10(8) CFU/ml, and unmodified milk, respectively, for 19 weeks. Faecal samples were taken at regular intervals to confirm gut transit, recovery of fed probiotics, and to assess the impact on the microflora. At sacrifice, the bowels were histologically scored. Cytokine production from Peyers' patches and splenocytes was measured in vitro by ELISA. RESULTS: Faecal recovery of probiotics was confirmed in all probiotic fed mice but not in controls. Colonic and caecal inflammatory scores were significantly decreased in both groups of probiotic fed mice ($p<0.05$). Proinflammatory cytokine production by Peyers' patches and splenocytes was significantly reduced in probiotic fed animals whereas transforming growth factor beta (TGF-beta) levels were maintained. CONCLUSION: Both Lactobacillus salivarius 433118 and Bifidobacterium infantis 35624 significantly attenuate colitis in this murine model. Attenuation of colitis is associated with a reduced ability to produce Th1-type cytokines systemically and mucosally, while levels of TGF-beta are maintained.

L17 ANSWER 2 OF 20 MEDLINE on STN
AN 2003073434 MEDLINE
TI Lactobacillus GG prevents recurrence of colitis in HLA-B27 transgenic rats after antibiotic treatment.
AU Dieleman L A; Goerres M S; Arends A; Sprengers D; Torrice C; Hoentjen F; Grenther W B; Sartor R B
SO GUT, (2003 Mar) 52 (3) 370-6.
Journal code: 2985108R. ISSN: 0017-5749.
AB BACKGROUND AND AIMS: Bacteroides vulgatus induces colitis in gnotobiotic HLA-B27 transgenic (TG) rats while broad spectrum antibiotics prevent and treat colitis in specific pathogen free (SPF) TG rats although disease recurs after treatment ends. Lactobacilli treat human pouchitis and experimental colitis. We investigated if Lactobacillus rhamnosus GG (L GG) can prevent colitis in TG rats monoassociated with B vulgatus and if L GG or Lactobacillus plantarum 299v (LP 299v) can treat established colitis in SPF TG rats and prevent recurrent disease after antibiotics were stopped. METHODS: Germfree B27 TG rats were monoassociated with B vulgatus for four weeks following two weeks of colonisation with L GG or no bacteria. SPF B27 TG rats received oral vancomycin and imipenem for two weeks, or water alone, followed by four weeks of treatment with oral L GG, LP 299v, or water only. Disease activity was quantified by blinded gross and histological scores, caecal myeloperoxidase (MPO) activity, and levels of interleukin (IL)-1 beta, tumour necrosis factor (TNF), transforming growth factor beta, and IL-10. RESULTS: L GG did not prevent colitis in B vulgatus co-associated TG rats or treat established disease in SPF rats. However, L GG but not LP 299v prevented colitis relapse in antibiotic treated rats with reduced gross and histological scores, caecal MPO, IL-1 beta, and TNF whereas caecal IL-10 was increased. CONCLUSIONS: L GG does not prevent colitis in gnotobiotic TG rats or treat established disease in SPF rats, but is superior to LP 299v in the prevention of recurrent colitis. These studies suggest that antibiotics and probiotic agents provide synergistic therapeutic

effects, perhaps mediated by altered immunomodulation with selective activity of different lactobacillus species.

L17 ANSWER 3 OF 20 MEDLINE on STN
AN 2002692695 MEDLINE
TI Variable response to probiotics in two models of experimental colitis in rats.
AU Shibolet Oren; Karmeli Fanny; Eliakim Rami; Swennen Erwin; Brigidi Patrizia; Gionchetti Paulo; Campieri Massimo; Morgenstern Sara; Rachmilewitz Daniel
SO INFLAMMATORY BOWEL DISEASES, (2002 Nov) 8 (6) 399-406.
Journal code: 9508162. ISSN: 1078-0998.
AB BACKGROUND AND AIM: Clinical and experimental data suggest an important role for intestinal microflora in the pathogenesis of inflammatory bowel disease, and probiotics have been shown to ameliorate pouchitis. We evaluated the effect of different preparations of probiotic bacteria on experimental colitis in rats. METHODS: Rats were treated daily intragastrically with two probiotic preparations, VSL#3 or strain GG (LGG), 7 days before induction of colitis and for another week thereafter. Colitis was induced by intracolonic administration of either dinitrobenzene sulfonic acid (DNBS) or iodoacetamide. Rats were killed 7 days after induction of colitis, the colon isolated, washed, weighed, lesion area measured, and mucosa processed for determination of myeloperoxidase (MPO) and nitric oxide synthase (NOS) activities and prostaglandin E2 (PGE2) generation. RESULTS: In rats cotreated with VSL#3 or LGG and iodoacetamide, there was a significant decrease in the lesion area, 98 +/- 37 mm and 142 +/- 43 mm, respectively, as compared with 342 +/- 66 mm in the control group. Colonic wet weight significantly decreased to 1.3 +/- 0.1 g/10 cm and 1.4 +/- 0.1 g/10 cm, respectively, as compared with 1.7 +/- 0.1 g/10 cm. There was also a significant decrease in PGE2 generation, MPO, and NOS activities in the VSL#3 and LGG treatment groups. Presence of VSL#3 bacteria in the rat's colon was confirmed by culture and polymerase chain reaction (PCR) amplification. Neither probiotic preparation had an effect on the extent of colonic damage in DNBS-induced colitis. CONCLUSION: Both VSL#3 and LGG significantly ameliorated colitis induced by the sulfhydryl-blocker iodoacetamide, but had no effect on the immune-mediated DNBS-induced colitis. The results suggest a possible role for sulfhydryl compounds in the protective effect of probiotic bacteria, and support their use in patients with inflammatory bowel disease.

L17 ANSWER 4 OF 20 MEDLINE on STN
AN 2002648782 MEDLINE
TI Probiotics and Crohn's disease.
AU Prantera C; Scribano M L
SO DIGESTIVE AND LIVER DISEASE, (2002 Sep) 34 Suppl 2 S66-7.
Journal code: 100958385. ISSN: 1590-8658.
AB Antibiotics are often employed in the treatment of Crohn's disease, with interesting results. However indiscriminate suppression of intestinal bacteria may be harmful and long-term use of antibiotics is burdened by side-effects and by the risk of developing bacterial resistance. Manipulation of enteric flora with probiotic compounds would be a possible and appealing alternative. First aim of our study has been to investigate the efficacy of this probiotic in reducing the endoscopic recurrence rate or in reducing the severity of recurrent lesions at 1 year after surgery. Secondary goal has

been the reduction of the clinical recurrence rate. Forty-five patients have been randomised to receive Lactobacillus rhamnosus strain GG or placebo for 12 months. The results have shown no differences in endoscopic and clinical remission between the two groups.

L17 ANSWER 5 OF 20 MEDLINE on STN
AN 2002648781 MEDLINE
TI Use of Lactobacillus-GG in paediatric Crohn's disease.
AU Guandalini S
SO DIGESTIVE AND LIVER DISEASE, (2002 Sep) 34 Suppl 2 S63-5.
Journal code: 100958385. ISSN: 1590-8658.
AB The potential role of luminal bacteria in initiating the abnormal immune response seen in inflammatory bowel disease is stressed by many observations. A defect in mucosal barrier function could allow luminal bacterial antigens to initiate the chronic relapsing inflammation in Crohn's disease. The potential role of luminal bacteria in initiating the abnormal immune response seen in inflammatory bowel disease is stressed by many observations. A pilot study to investigate the possible effect of Lactobacillus GG in children with active Crohn's disease was conducted. Four male patients were enrolled, median age 14.5 years (range 10-18). In terms of clinical outcome, the patients showed significant improvement. In three patients on Lactobacillus GG, it was possible to taper the dose of steroids. Thus, although our data are obviously very preliminary, Lactobacillus GG appears to be effective in improving the clinical status of children with Crohn's disease. A multicentre study is currently being carried out in 7 US University centres in a randomized, double-blind, placebo-controlled fashion to establish the efficacy of this probiotic in children with Crohn's disease.

L17 ANSWER 6 OF 20 MEDLINE on STN
AN 2002418072 MEDLINE
TI Ineffectiveness of probiotics in preventing recurrence after curative resection for Crohn's disease: a randomised controlled trial with Lactobacillus GG.
AU Prantera C; Scribano M L; Falasco G; Andreoli A; Luzi C
SO GUT, (2002 Sep) 51 (3) 405-9.
Journal code: 2985108R. ISSN: 0017-5749.
AB BACKGROUND AND AIMS: Experimental studies have shown that luminal bacteria may be involved in Crohn's disease. Probiotics are a possible alternative to antibiotics. The aim of this randomised placebo controlled study was to determine if Lactobacillus GG, given by mouth for one year, could prevent Crohn's recurrent lesions after surgery or to reduce their severity. METHODS: Patients operated on for Crohn's disease in whom all of the diseased gut had been removed were randomly allocated to receive 12 billion colony forming units of Lactobacillus or identical placebo for one year. Ileocolonoscopy was performed at the end of the trial or at the onset of symptoms. Endoscopic recurrence was defined as grade 2 or higher of Rutgeerts scoring system. RESULTS: Eight of 45 patients were excluded from the trial (three for non-compliance and five for protocol violations). Clinical recurrence was ascertained in three (16.6%) patients who received Lactobacillus and in two (10.5%) who received placebo. Nine of 15 patients in clinical remission on Lactobacillus (60%) had endoscopic recurrence compared with six of 17 (35.3%) on placebo (p=0.297). There were no significant differences in the

severity of the lesions between the two groups. CONCLUSIONS: Lactobacillus GG seems neither to prevent endoscopic recurrence at one year nor reduce the severity of recurrent lesions.

L17 ANSWER 7 OF 20 MEDLINE on STN
AN 2002172766 MEDLINE
TI Lactobacillus plantarum 299V in the treatment and prevention of spontaneous colitis in interleukin-10-deficient mice.
AU Schultz Michael; Veltkamp Claudia; Dieleman Levinus A; Grenther Wetonia B; Wyrick Pricilla B; Tonkonogy Susan L; Sartor R Balfour
SO INFLAMMATORY BOWEL DISEASES, (2002 Mar) 8 (2) 71-80.
Journal code: 9508162. ISSN: 1078-0998.
AB Interleukin (IL)-10-deficient (IL-10-/-) mice develop colitis under specific pathogen-free (SPF) conditions and remain disease free if kept sterile (germ free [GF]). We used four different protocols that varied the time-points of oral administration of Lactobacillus plantarum 299v (L. plantarum) relative to colonization with SPF bacteria to determine whether L. plantarum could prevent and treat colitis induced by SPF bacteria in IL-10-/- mice and evaluated the effect of this probiotic organism on mucosal immune activation. Assessment of colitis included blinded histologic scores, measurements of secreted colonic immunoglobulin isotypes, IL-12 (p40 subunit), and interferon (IFN)-gamma production by anti-CD3-stimulated mesenteric lymph node cells. Treating SPF IL-10-/- mice with L. plantarum attenuated previously established colonic inflammation as manifested by decreased mucosal IL-12, IFN-gamma, and immunoglobulin G2a levels. Colonizing GF animals with L. plantarum and SPF flora simultaneously had no protective effects. Gnotobiotic IL-10-/- mice monoassociated with L. plantarum exhibited mild immune system activation but no colitis. Pretreatment of GF mice by colonization with L. plantarum, then exposure to SPF flora and continued probiotic therapy significantly decreased histologic colitis scores. These results demonstrate that L. plantarum can attenuate immune-mediated colitis and suggest a potential therapeutic role for this agent in clinical inflammatory bowel diseases.

L17 ANSWER 8 OF 20 MEDLINE on STN
AN 2002108292 MEDLINE
TI Mucosal barrier function and the commensal flora.
AU Kennedy R J; Kirk S J; Gardiner K R
SO GUT, (2002 Mar) 50 (3) 441-2.
Journal code: 2985108R. ISSN: 0017-5749.

L17 ANSWER 9 OF 20 MEDLINE on STN
AN 2001546455 MEDLINE
TI Is lactobacillus GG helpful in children with Crohn's disease? Results of a preliminary, open-label study.
AU Gupta P; Andrew H; Kirschner B S; Guandalini S
SO JOURNAL OF PEDIATRIC GASTROENTEROLOGY AND NUTRITION, (2000 Oct) 31 (4) 453-7.
Journal code: 8211545. ISSN: 0277-2116.
AB BACKGROUND: Lactobacillus GG is a safe probiotic bacterium known to transiently colonize the human intestine. It has been found to be useful in treatment of several gastrointestinal conditions characterized by increased gut permeability. In the current study, the efficacy of Lactobacillus GG was investigated in children with Crohn's disease. METHODS: In this open-label pilot evaluation

viewed as a necessary preliminary step for a possible subsequent randomized placebo-controlled trial, four children with mildly to moderately active Crohn's disease were given Lactobacillus GG (10(10) colony-forming units [CFU]) in enterocoated tablets twice a day for 6 months. Changes in intestinal permeability were measured by a double sugar permeability test. Clinical activity was determined by measuring the pediatric Crohn's disease activity index. RESULTS: There was a significant improvement in clinical activity 1 week after starting Lactobacillus GG, which was sustained throughout the study period. Median pediatric Crohn's disease activity index scores at 4 weeks were 73% lower than baseline. Intestinal permeability improved in an almost parallel fashion. CONCLUSIONS: Findings in this pilot study show that Lactobacillus GG may improve gut barrier function and clinical status in children with mildly to moderately active, stable Crohn's disease. Randomized, double-blind, placebo-controlled trials are warranted for a final assessment of the efficacy of Lactobacillus GG in Crohn's disease.

L17 ANSWER 10 OF 20 MEDLINE on STN
AN 2001479051 MEDLINE
TI Probiotic bacteria enhance murine and human intestinal epithelial barrier function.
AU Madsen K; Cornish A; Soper P; McKaigney C; Jijon H; Yachimec C; Doyle J; Jewell L; De Simone C
SO GASTROENTEROLOGY, (2001 Sep) 121 (3) 580-91.
Journal code: 0374630. ISSN: 0016-5085.
AB BACKGROUND & AIMS: The probiotic compound, VSL#3, is efficacious as maintenance therapy in pouchitis and ulcerative colitis. The aim of this study was to determine the efficacy of VSL#3 as a primary therapy in the treatment of colitis in the interleukin (IL)-10 gene-deficient mouse. Mechanisms of action of VSL#3 were investigated in T(84) monolayers. METHODS: IL-10 gene-deficient and control mice received 2.8 x 10(8) colony-forming units per day of VSL#3 for 4 weeks. Colons were removed and analyzed for cytokine production, epithelial barrier function, and inflammation. VSL#3 or conditioned media was applied directly to T(84) monolayers. RESULTS: Treatment of IL-10 gene-deficient mice with VSL#3 resulted in normalization of colonic physiologic function and barrier integrity in conjunction with a reduction in mucosal secretion of tumor necrosis factor alpha and interferon gamma and an improvement in histologic disease. In vitro studies showed that epithelial barrier function and resistance to Salmonella invasion could be enhanced by exposure to a proteinaceous soluble factor secreted by the bacteria found in the VSL#3 compound. CONCLUSIONS: Oral administration of VSL#3 was effective as primary therapy in IL-10 gene-deficient mice, and had a direct effect on epithelial barrier function.

L17 ANSWER 11 OF 20 MEDLINE on STN
AN 1999238884 MEDLINE
TI Lactobacillus species prevents colitis in interleukin 10 gene-deficient mice.
AU Madsen K L; Doyle J S; Jewell L D; Tavernini M M; Fedorak R N
SO GASTROENTEROLOGY, (1999 May) 116 (5) 1107-14.
Journal code: 0374630. ISSN: 0016-5085.
AB BACKGROUND & AIMS: Intestinal luminal microflora, or their products, are likely an important initiating factor in the pathogenesis of

inflammatory bowel disease. The aim of this study was to determine the role of colonic aerobic luminal bacteria and Lactobacillus species in the development of colitis in interleukin (IL)-10 gene-deficient mice. METHODS: Intestine from 2-16-week-old mice was scored histologically and cultured for bacteria. Lactobacillus sp. repopulation of the colonic lumen was achieved via daily rectal delivery of Lactobacillus reuteri or oral lactulose therapy. RESULTS: At 2 weeks of age, IL-10 gene-deficient mice showed no colonic injury but did display abnormal colonic bacterial colonization with increased colonic mucosal aerobic adherent and translocated bacteria in conjunction with reduced Lactobacillus sp. levels. In association with the abnormal colonic bacterial colonization, colitis developed by 4 weeks of age. Restoring Lactobacillus sp. to normal levels reduced levels of colonic mucosal adherent and translocated bacteria and attenuated the development of the colitis. CONCLUSIONS: In the neonatal period, IL-10 gene-deficient mice have decreased levels of colonic Lactobacillus sp. and an increase in colonic mucosal adherent and translocated bacteria. Normalizing Lactobacillus sp. levels reduced colonic mucosal adherent and translocated bacteria and prevented colitis.

L17 ANSWER 12 OF 20 MEDLINE on STN
AN 97016059 MEDLINE
TI Promotion of IgA immune response in patients with Crohn's disease by oral bacteriotherapy with Lactobacillus GG.
AU Malin M; Suomalainen H; Saxelin M; Isolauri E
SO ANNALS OF NUTRITION AND METABOLISM, (1996) 40 (3) 137-45.
Journal code: 8105511. ISSN: 0250-6807.
AB The effect of oral bacteriotherapy with human Lactobacillus casei strain GG (10(10) colony-forming units twice daily for 10 days) was investigated in Crohn's disease and in juvenile chronic arthritis which are chronic inflammatory diseases associated with impaired mucosal barrier function. During oral bacteriotherapy, the gut immune response was indirectly assessed by solid-phase enzyme-linked immunoassay in 14 children with Crohn's disease, in 9 with juvenile chronic arthritis, and in 7 controls. The immunostimulatory effect of Lactobacillus GG was specific for Crohn's disease, irrespective of its activity: the mean (95% confidence interval) number of specific antibody secreting cells in the IgA class to beta-lactoglobulin increased significantly from 0.2 (0.04-1.3) to 1.4 (0.3-6.0)/10(6) cells and to casein from 0.3 (0.1-1.4) to 1.0 (0.2-4.8)/10(6) cells. The results indicate that orally administered Lactobacillus GG has the potential to increase the gut IgA immune response and thereby to promote the gut immunological barrier. Consequently, Lactobacillus GG could provide an adjunct nutritional therapy for Crohn's disease.

L17 ANSWER 13 OF 20 MEDLINE on STN
AN 88093348 MEDLINE
TI Successful treatment of relapsing Clostridium difficile colitis with Lactobacillus GG.
AU Gorbach S L; Chang T W; Goldin B
SO LANCET, (1987 Dec 26) 2 (8574) 1519.
Journal code: 2985213R. ISSN: 0140-6736.

L17 ANSWER 14 OF 20 MEDLINE on STN
AN 78138849 MEDLINE
TI [Use of biphicol in the treatment of chronic colitis].

Opyt primrneniia bifikola pri lechenii bol'nykh chronicheskim kolitom.
AU Malygin A G; Pospelova V V; Rakhimova N G; Voroshilova N N; Nizov A A
SO SOVETSKAIA MEDITSINA, (1978 Jan) (1) 117-21.
Journal code: 0404525. ISSN: 0038-5077.

L17 ANSWER 15 OF 20 MEDLINE on STN
AN 78119206 MEDLINE
TI [Use of bifidumbacterin for prevention of staphylococcal infections in newborn infants].
Opyt primeneniia bifidumbakterina dlia profilaktiki stafilokokkovykh zabolevanii u novorozhdennykh.
AU Astakhov I I
SO VOPROSY OKHRANY MATERINSTVA I DETSTVA, (1978 Jan) 23 (1) 75-7.
Journal code: 0416600. ISSN: 0042-8825.

L17 ANSWER 16 OF 20 MEDLINE on STN
AN 77180307 MEDLINE
TI [Effect of bificol on the intestinal microflora of chronic colitis patients working in antiboitic prodiction].
Vliianie bifikola na mikrofloru kishechnika bol'nykh knronicheskim kolitom u usloviiakh proizvodstva antibiotikov.
AU Vil'shanskaia F L; Mazitova O P; Shteinberg G B; Pospelova V V; Rakhimova N G
SO ANTIBIOTIKI, (1977) 22 (2) 181-4.
Journal code: 0375020. ISSN: 0003-5637.
AB Data on the use of bificol, a new Soviet preparation, and its effect on the intestine microflora of patients with chronic colitis occupied in production of penicillin are presented. It was shown that by the 28th day of the preparation use the level of the intestine bacteria in the patients' intestine reliably increased. The number of immobile strains decreased from 67.6 to 36.6 per cent. Bifidoflora normalized by the 14th day of the treatment. Some clinical inprovement, i.e. stool normalization, lessening of the stomach pain, increased appetite were observed by the 4th--5th day of the treatment with bificol. On the basis of the microbiological and clinical data it was shown that treatment of the patients with chronic colitis in antibiotic production should continue for at least 28 days and in individual cases for longer periods of time. It is recommended to use the preparation in 10 doses a day divided into 2 parts.

L17 ANSWER 17 OF 20 MEDLINE on STN
AN 77174918 MEDLINE
TI [Treatment of chronic enterocolitis with dry lactobacterin].
K lecheniiu khronicheskogo enterokolita sukhim laktobakterin.
AU Levin A A; Tsimmerman Ia S; Krasik E L; Gal'ianova G A
SO SOVETSKAIA MEDITSINA, (1977 Feb) (2) 109-13.
Journal code: 0404525. ISSN: 0038-5077.

L17 ANSWER 18 OF 20 MEDLINE on STN
AN 77174917 MEDLINE
TI [Use of dry bifidumbacterin in the complex treatment of chronic inflammatory diseases of the large intestine].
Primenenie sukhovo bifidumbakterina v kompleksnom lechenii khronicheskikh vospalitel'nykh zabolevanii tolstoi kishki.
AU Iukhvidov Zh M; Semenova L P; Kuznetsov G G; Grigor'eva G A;

Goncharova G P
SO SOVETSKAIA MEDITSINA, (1977 Feb) (2) 104-9.
Journal code: 0404525. ISSN: 0038-5077.

L17 ANSWER 19 OF 20 MEDLINE on STN
AN 73081874 MEDLINE
TI Patterns of bile acids and microflora in the human small intestine. II. Microflora.
AU Mallory A; Savage D; Kern F Jr; Smith J G
SO GASTROENTEROLOGY, (1973 Jan) 64 (1) 34-42.
Journal code: 0374630. ISSN: 0016-5085.

L17 ANSWER 20 OF 20 MEDLINE on STN
AN 68403297 MEDLINE
TI [Use of nicodin and streptomycin in the therapy of chronic colitis and enterocolitis].
Primenenie nikodina i streptomitsina v terapii khronicheskikh kolitov i enterokolitov.
AU Ekisenina N I; Ivanova L M; Kudinova T I
SO ANTIBIOTIKI, (1967 Dec) 12 (12) 1109-14.
Journal code: 0375020. ISSN: 0003-5637.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, CANCERLIT, PHIC, PHIN, TOXCENTER' ENTERED AT 14:05:07 ON 09 OCT 2003)
L18 4 S L9
L19 4 DUP REM L18 (0 DUPLICATES REMOVED)

L19 ANSWER 1 OF 4 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 2003047724 EMBASE
TITLE: Modification of **intestinal** flora in the treatment of inflammatory bowel **disease**.
AUTHOR: Kanauchi O.; Mitsuyama K.; Araki Y.; Andoh A.
CORPORATE SOURCE: O. Kanauchi, Nutrient Food and Feed Division, Kirin Brewery Co. Ltd., 10-1-2 Shinkawa Chuo-ku, Tokyo 104-8288, Japan. kanauchio@kirin.co.jp
SOURCE: Current Pharmaceutical Design, (2003) 9/4 (333-346).
Refs: 92
ISSN: 1381-6128 CODEN: CPDEFP
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 004 Microbiology
030 Pharmacology
037 Drug Literature Index
048 Gastroenterology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Because the **intestinal** microflora play an important role in the development of inflammatory bowel **disease** (IBD), there is currently some interest in the manipulation of the composition of the microflora towards a potentially more remedial community. This review summarizes the clinical and experimental efficacy of the manipulation of microflora by the use of prebiotics, probiotics, synbiotics, and antibiotics in IBD. Prebiotics, defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth or activity of one or a limited number of bacterial species already resident in the colon,

can modulate the colonic microbiota by increasing the number of specific bacteria and thus changing the composition of the microbiota. Prebiotics for IBD include lactosucrose, oligofructose, inulin, bran, psyllium, and germinated barley foodstuff (GBF). GBF, which mainly consists of dietary fiber and glutamine-rich protein, is a prebiotic foodstuff for **ulcerative colitis**.
GBF has shown to be converted into a preferential nutrient for colonocytes through Eubacterium and Bifidobacterium and also inactivate nuclear factor kappa B (NFkB). Moreover, it exhibits a potent waterholding capacity and bile-acid binding capacity.
Probiotics, which are microbial food supplements that beneficially affect the host by improving the **intestinal** microbial balance, have been used to change the composition of colonic microbiota. The approaches for IBD include VSL#3, Nissle1917, Clostridium butyricum and Bifidobacterium-fermented milk. Use of **Lactococci** secreting IL-10 provides excellent results. The combination of prebiotics and probiotics in a synbiotic has not been studied in IBD but is promising. The use of antibiotics continues to be of interest. Although these strategies hold great promise and appear to be useful in some settings, more clinical study is needed to firmly establish the relevance of these therapies.

L19 ANSWER 2 OF 4 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2002429708 EMBASE
TITLE: Probiotics and health: New facts and ideas.
AUTHOR: Marteau P.; Seksik P.; Jian R.
CORPORATE SOURCE: P. Marteau, Gastroenterology Department, European Hospital Georges Pompidou, Ass. Publ. Hop. Paris/Paris V Univ., Paris, France. philippe.marteau@egp.ap-hop-paris.fr
SOURCE: Current Opinion in Biotechnology, (1 Oct 2002) 13/5 (486-489).
Refs: 30
ISSN: 0958-1669 CODEN: CUOBE3
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 004 Microbiology
013 Dermatology and Venereology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
048 Gastroenterology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Many trials on probiotics are now published that use established methods to demonstrate their clinical efficacy. Convincing progress has been made in the field of inflammatory bowel **disease** and allergy prevention in infants. Experimental studies show clear differences (and even sometimes opposite effects) between apparently closely related probiotics and suggest new mechanisms for the observed effects, such as immunostimulation by bacterial DNA and interaction with Toll-like receptors and dendritic cells in the **gastrointestinal** tract.

L19 ANSWER 3 OF 4 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2002177788 EMBASE
TITLE: Probiotics and immune response.

AUTHOR: Blum S.; Haller D.; Pfeifer A.; Schiffrin E.J.
CORPORATE SOURCE: S. Blum, Immunology/Bioscience, Nestle Research Center, Vers-chez-les-Blanc, 1000 Lausanne 26, Switzerland. stephanie.blum-sperisen@rdls.nestle.com
SOURCE: Clinical Reviews in Allergy and Immunology, (2002) 22/3 (287-309).
Refs: 109
ISSN: 1080-0549 CODEN: CRVADD
COUNTRY: United States
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LANGUAGE: English

L19 ANSWER 4 OF 4 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
ACCESSION NUMBER: 2001-138142 [14] WPIDS
DOC. NO. CPI: C2001-040684
TITLE: Recombinant **Lactococcus lactis** for delivering a **trefoil** peptide useful for treating acute or chronic **gastrointestinal** inflammatory **diseases** or **disorders**, e.g. acute or **ulcerative colitis**, acute flare-ups of **Crohn**'s **disease**.
DERWENT CLASS: B04 C03 D16
INVENTOR(S): HANS, W C; REMAUT, E R; STEIDLER, L
PATENT ASSIGNEE(S): (VLAA-N) VLAAMS INTERUNIVERSITAIR INST BIOTECHNOG
COUNTRY COUNT: 94
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001002570	A1	20010111	(200114)*	EN	59
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW					
AU 2000066892	A	20010122	(200125)		
EP 1194554	A1	20020410	(200232)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
JP 2003504025	W	20030204	(200320)		48

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001002570	A1	WO 2000-EP6343	20000705
AU 2000066892	A	AU 2000-66892	20000705
EP 1194554	A1	EP 2000-954434	20000705
		WO 2000-EP6343	20000705
JP 2003504025	W	WO 2000-EP6343	20000705
		JP 2001-508342	20000705

Searcher : Shears 308-4994

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2000066892	A	Based on	WO 2001002570
EP 1194554	A1	Based on	WO 2001002570
JP 2003504025	W	Based on	WO 2001002570

PRIORITY APPLN. INFO: EP 1999-870143 19990705
AN 2001-138142 [14] WPIDS
AB WO 200102570 A UPAB: 20010312
NOVELTY - A recombinant **Lactococcus lactis** capable of delivering a **trefoil** peptide in vivo, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a method for the delivery of **trefoil** peptide to the gastrointestinal tract by administering the recombinant microorganism;

(2) methods of treating **gastric** and/or **intestinal diseases** and/or **disorders**, or lesions caused by these **disorders**;

(3) a method for producing a microorganism able to deliver **trefoil** peptide by transforming a microorganism with a recombinant vector carrying a **trefoil** peptide coding sequence under the control of a promoter and a secretion signal sequence; and

(4) a recombinant vector comprising a **trefoil** peptide coding sequence under the control of a promoter sequence and a secretion signal sequence.

ACTIVITY - Antiinflammatory; gastrointestinal; anti-**ulcer**.

The effect of **trefoil** peptides expressed from L. **lactis** was tested in mice suffering from acute **colitis**. 21 female Balb/c mice received 5% dextrane sodium sulfate to induce acute **colitis**. For therapeutic purposes, mice were orally inoculated daily with 100 mu l bacterial suspension (108 cells) from day 1-7. 6 Mice were inoculated with MG1363 (pTREX1) cells, 6 were inoculated with MG1363 (pT1mTFF1) cells, and 3 served as controls. On day 8 after induction of **colitis**, mice were sacrificed and examined for immunological and histological testing. Results showed that there was a clear decrease of inflammation upon inoculation of mice with L. **lactis** cells producing **trefoil** peptides. There was a 65% reduction of inflammation in mice that received (pT1mTFF1)-transformed L. **lactis** cells. Inflammatory infiltration and epithelial damage in the distal part of the colon were significantly decreased following inoculation with recombinant L. **lactis** strains which secrete mTFF1 polypeptide.

MECHANISM OF ACTION - Peptide therapy.

USE - The recombinant microorganism is useful for the manufacturing an agent for the delivery of a **trefoil** peptide to the **gastrointestinal** tract, and for treating **gastric** or **intestinal diseases** or **disorders**, or lesions caused by **gastric** or **intestinal diseases** or **disorders**. The microorganism may also be used for preparing medicament to be used for treating **gastric** and /or **gastrointestinal** **diseases** or **disorders**, acute

gastrointestinal inflammatory **diseases** (e.g., acute **colitis**, acute flare-ups of **Crohn**'s **diseases**, or **ulcerative colitis**), and chronic and spontaneously recurring **diseases** of the **gastrointestinal** tract comprising **Crohn**'s **disease** (**enteritis regionalis**) and **ulcerative colitis** (**colitis ulcerosa**) (all claimed). **Disease** states which can be treated by the method or compositions comprising the recombinant microorganism or **trefoil** peptides, include **disorders** of and damage to the alimentary canal, including the mouth, esophagus, stomach and large and small **intestine**, as well as for the protection and treatment of tissues that lie outside the alimentary canal.
Dwg.0/8

(FILE 'HCAPLUS, MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO, CANCERLIT, PHIC, PHIN, TOXCENTER' ENTERED AT 14:06:30 ON 09 OCT 2003)

```
L20         217 S "HANS W"?/AU
L21         100 S ("STIEDLER L"? OR "STEIDLER L"?)/AU
L22         260 S "REMAUT E"?/AU
L23           9 S L20 AND L21 AND L22
L24           9 S L20 AND (L21 OR L22)
L25          70 S L21 AND L22
L26          25 S (L20 OR L21 OR L22 OR L25) AND L8
L27          25 S L23 OR L24 OR L26
L28          12 DUP REM L27 (13 DUPLICATES REMOVED)
```

Author (s)

L28 ANSWER 1 OF 12 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2003264059 EMBASE
TITLE: Biological containment of genetically modified **Lactococcus lactis** for intestinal delivery of human interleukin 10.
AUTHOR: **Steidler L.**; Neirynck S.; Huyghebaert N.; Snoeck V.; Vermeire A.; Goddeeris B.; Cox E.; Remon J.P.; **Remaut E.**
CORPORATE SOURCE: L. Steidler, Dept. of Molec. Biomedical Research, Vlaams Interuniv. Inst. V. Biotech., Ghent University, KL. Ledeganckstraat 35, B-9000 Ghent, Belgium. l.steidler@ucc.ie
SOURCE: Nature Biotechnology, (1 Jul 2003) 21/7 (785-789). Refs: 36
ISSN: 1087-0156 CODEN: NABIF
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Genetically modified **Lactococcus lactis** secreting interleukin 10 provides a therapeutic approach for inflammatory bowel disease. However, the release of such genetically modified organisms through clinical use raises safety concerns. In an effort to address this problem, we replaced the thymidylate synthase gene thyA of L. **lactis** with a synthetic human IL10 gene. This thyA(-) hIL10(+) L. **lactis** strain produced human IL-10 (hIL-10), and when deprived of thymidine or thymine, its

viability dropped by several orders of magnitude, essentially preventing its accumulation in the environment. The biological containment system and the bacterium's capacity to secrete hIL-10 were validated in vivo in pigs. Our approach is a promising one for transgene containment because, in the unlikely event that the engineered L. **lactis** strain acquired an intact thyA gene from a donor such as L. **lactis** subsp. cremoris, the transgene would be eliminated from the genome.

L28 ANSWER 2 OF 12 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:612809 HCAPLUS
TITLE: In situ delivery of therapeutic proteins by recombinant **Lactococcus lactis**
AUTHOR(S): **Steidler, Lothar**; Neirynck, Sabine
CORPORATE SOURCE: University College Cork, Cork, Ire.
SOURCE: Journal of Microbiology (Seoul, Republic of Korea) (2003), 41(2), 63-72
CODEN: JOMIFG; ISSN: 1225-8873
PUBLISHER: Microbiological Society of Korea
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Chronic inflammatory bowel disease (IBD) such as **Crohn**'s disease or **ulcerative colitis**, affects around 2 in every 1000 individuals in western countries and its incidence, particularly amongst children, is increasing. IBD shows extreme morbidity with impact on all aspects of quality of life. If left untreated, IBD can lead to death. Conventional treatment of IBD involves powerful immunosuppressive chemotherapies and surgical intervention. Long-term anti-inflammatory medication is required and so patients are often subject to a spectrum of unpleasant side effects. Interleukin-10 (IL-10) is a cytokine that acts to suppress inflammation. When however administered by injection, the high levels of IL-10 that are distributed throughout the body also lead to side effects. **Lactococcus lactis** can be genetically engineered to secrete biol. active cytokines. When applied to the mucosa, these L. **lactis** can actively deliver such cytokines. By use of this principle we developed a new therapeutic approach for IBD. Administration of L. **lactis** that secretes murine IL-10 cures and prevents IBD in mice. The use of the engineered L. **lactis** gets around the problem of delivering IL-10, allowing dramatic reduction of the ED. A sincere concern exists about the possible dangers of uncontrolled, deliberate release of genetically modified microorganisms, such as could occur following application in healthcare. We engaged in the establishment of adequate means for biol. growth control of engineered L. **lactis** by targeted gene exchange between thyA and hIL-10.
REFERENCE COUNT: 72 THERE ARE 72 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L28 ANSWER 3 OF 12 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 1
ACCESSION NUMBER: 2002:869090 HCAPLUS
DOCUMENT NUMBER: 137:364400
TITLE: **Lactococcus** strain containing human interleukin 10 gene and an inactivated thyA gene, and its use as delivery vehicle for treatment of inflammatory bowel disease

INVENTOR(S): **Steidler, Lothar**
PATENT ASSIGNEE(S): Vlaams Interuniversitair Instituut Voor Biotechnologie VZW, Belg.
SOURCE: PCT Int. Appl., 65 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002090551	A2	20021114	WO 2002-EP4942	20020503
WO 2002090551	A3	20030327		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: EP 2001-201631 A 20010503
EP 2001-204785 A 20011207

AB The invention provides a strain of **Lactococcus** (such as L. **lactis**) that is defective in the thymidylate synthase gene (thyA), and use of said strain as a host for genetic transformation. The invention also provides plasmid vectors (pOThy11, pOThy12, pOThy15 and pOThy16) containing nucleic acids encoding human interleukin 10, and use of said plasmids in transforming the L. **lactis** thyA defective strain. The invention relates that the inactivated thyA gene of L. **lactis** has been replaced by the human IL-10 gene. The invention further provides the use of said transformed L. **lactis**, containing human IL-10 gene, as a delivery vehicle for treatment of inflammatory bowel disease. The invention related that the recombinant L. **lactis** strain has environmentally limited growth and viability, hence is safe for recombinant production of therapeutic/prophylactic mols. The invention specifically demonstrated that the recombinant human IL-10 produced by transgenic L. **lactis** strain LL108 showed full biol. activity. The invention also demonstrated that transformed L. **lactis** (Thy12) passed the intestine of mice at the same speed as the wild-type strain, and that Thy12 requires thymidine for growth. The invention also presented the DNA and amino acid sequences of gene thyA, including the 5'-flank and 3'-flank nucleotide sequences, which are important areas in genetic engineering of **Lactococcus** strains.

L28 ANSWER 4 OF 12 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 2
ACCESSION NUMBER: 2002:788141 HCAPLUS
DOCUMENT NUMBER: 139:41495
TITLE: In situ delivery of cytokines by genetically engineered **Lactococcus lactis**
AUTHOR(S): **Steidler, Lothar**
CORPORATE SOURCE: Department of Molecular Biology, VIB-Ghent

University, Ghent, B-9000, Belg.
SOURCE: Antonie van Leeuwenhoek (2002), 82(1-4), 323-331
CODEN: ALJMAO; ISSN: 0003-6072
PUBLISHER: Kluwer Academic Publishers
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review and discussion. The development of novel approaches that allow for accurate targeting of therapeutics to the bowel mucosa is a priority in the research on inflammatory bowel disease. The author engineered **Lactococcus lactis** to secrete soluble, fully active, correctly processed cytokines. The author has used these live, recombinant strains for the in situ delivery of mouse interleukin (mIL)-2, -6 and -10 at airway mucosa or mucosa of the colon. Strains that secrete mIL-2 or mIL-6 and produce TTFC intracellular show a higher level of anti-TTFC induction in mice following intranasal inoculation. The author showed that mIL-10 producing L. **lactis** can prevent and cure enterocolitis in mice. The daily ingestion of this strain leads to the prevention of **colitis** in IL-10 -/- 129 Sv/Ev mice. The repeated addition of DSS to the drinking water of Balb/c mice leads to the induction of chronic **colitis** with a typical mean histol. score of five points. Subsequent daily treatment with 108 IL-10 producing L. **lactis** reduced the inflammation to a score of approx. 1 in 40% of the treated mice, which is a status equal to that of healthy control mice. Most other animals from the treated group only showed minor patchy remnants of the inflammation. Killing of the IL-10 producing bacteria by UV irradiation immediately prior to inoculation abrogates this therapeutic effect. Therefore it can be attributed to the active in vivo delivery of IL-10. The authors has further documented this by demonstrating in situ de novo synthesis of IL-10 in the colon of IL-10 -/- mice.

REFERENCE COUNT: 65 THERE ARE 65 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L28 ANSWER 5 OF 12 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 3
ACCESSION NUMBER: 2001:31647 HCAPLUS
DOCUMENT NUMBER: 134:95499
TITLE: Transformed **Lactococcus** or **Lactobacillus** containing recombinant plasmid vectors encoding trefoil peptide TFF1 used for treatment of **gastric** and/or **intestinal disorders**
INVENTOR(S): **Hans, Wolfgang Christian; Steidler, Lothar; Remaut, Erik Rene**
PATENT ASSIGNEE(S): Vlaams Interuniversitair Instituut voor Biotechnologie, Belg.
SOURCE: PCT Int. Appl., 44 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001002570	A1	20010111	WO 2000-EP6343	20000705

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

EP 1194554 A1 20020410 EP 2000-954434 20000705
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

JP 2003504025 T2 20030204 JP 2001-508342 20000705

PRIORITY APPLN. INFO.: EP 1999-870143 A 19990705
WO 2000-EP6343 W 20000705

AB The invention provides recombinant plasmid vectors containing DNA sequences encoding the mature mouse trefoil peptide TFF1 (originally named pS2), under the control of a suitable promoter. The invention also provides the use of said recombinant plasmid vector in transforming **Lactococcus** or **Lactobacillus** species, and methods used for producing recombinant TFF1 in said bacteria. The invention further provides a pharmaceutical composition comprising the recombinant **Lactococcus** or **Lactobacillus**. Still further, the invention provides for the use of recombinant **Lactococcus** or **Lactobacillus**: (1) in treatment of **gastric** and/or **intestinal diseases** or **disorders**, such as acute **colitis**, acute flare ups of **Crohn**'s **disease** and **ulcerative colitis**, and (2) in treatment or inhibition of lesions caused by such **diseases**. Finally, the invention provides the DNA sequence of the recombinant plasmid vectors, pL2mTFF1v1, pT1mTFF1 and pPICmTFF1, which encode part or all of the mouse mature TFF1 peptide. In the example section, the invention showed that mice, suffering from acute **colitis**, showed a significant reduction of intestinal inflammation when inoculated with recombinant L. **lactis** containing plasmid pT1mTFF1. The invention also presented the construction of plasmid pPICmTFF1, which was used to recombinantly produce TFF1 using Pichia pastoris.

REFERENCE COUNT: 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L28 ANSWER 6 OF 12 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 4
ACCESSION NUMBER: 2001:827770 HCAPLUS
DOCUMENT NUMBER: 137:4517
TITLE: Microbiological and immunological strategies for treatment of inflammatory bowel disease
AUTHOR(S): **Steidler, Lothar**
CORPORATE SOURCE: Department of Molecular Biology, Flanders Interuniversity Institute for Biotechnology, Ghent University, Ghent, B-9000, Belg.
SOURCE: Microbes and Infection (2001), 3(13), 1157-1166
CODEN: MCINFS; ISSN: 1286-4579
PUBLISHER: Editions Scientifiques et Medicales Elsevier
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review discusses therapy for inflammatory bowel diseases. Chronic

inflammatory bowel diseases such as **Crohn**'s disease or **ulcerative colitis**, affect around 1 in every 1000 individuals in western countries. They probably result from an inappropriate reaction towards the commensal microflora and are currently treated with anti-inflammatory drugs or surgery. Novel strategies aim at blocking lymphocyte recruitment and activation, improved targeting of therapeutics and modification of gut microflora.

REFERENCE COUNT: 100 THERE ARE 100 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L28 ANSWER 7 OF 12 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 5
ACCESSION NUMBER: 2001:892472 HCAPLUS
DOCUMENT NUMBER: 137:114304
TITLE: Lactoccocus **lactis**, a tool for the delivery of the therapeutic proteins. Treatment of IBD
AUTHOR(S): **Steidler, Lothar**
CORPORATE SOURCE: Department of Molecular Biology, VIB, Ghent University, Ghent, B-9000, Belg.
SOURCE: TheScientificWorld [online computer file] (2001), 1, 216-217
CODEN: THESAS; ISSN: 1532-2246
URL: http://216.25.253.202/TSWJaudit/pdf/2001.37.pdf
PUBLISHER: TheScientificWorld, Inc.
DOCUMENT TYPE: Journal; General Review; (online computer file)
LANGUAGE: English
AB A review. A novel approach for the treatment of chronic **intestinal** inflammation in mice and has the potential for treating inflammatory bowel **disease** (IBD) was developed. This novel approach involves the use of genetically modified L. **lactis**, which can cure and prevent IBD by releasing interleukin-10 (IL-10), a cytokine produced by the immune cells of the body and suppresses inflammation. In an experiment which investigated whether the orally administered IL-10 producing L. **lactis** could be effective in reversing the inflammation related with exptl. **colitis**, the IBD-induced mice were cured by the daily feeding of mIL-10 producers L. **lactis**. An accurate dosage and timing during treatment can easily be obtained with L. **lactis**, and this approach is intrinsically very cost effective.
REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L28 ANSWER 8 OF 12 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:678893 HCAPLUS
DOCUMENT NUMBER: 136:215017
TITLE: In situ delivery of cytokines at mucosal surfaces by genetically engineered **Lactococcus lactis**
AUTHOR(S): **Steidler, Lothar**
CORPORATE SOURCE: Department of Molecular Biology, Flanders Inter-University Institute for Biotechnology and the University of Gent, Ghent, B-9000, Belg.
SOURCE: NATO Science Series, Series I: Life and

Behavioural Sciences (2001), 338(Novel Processes and Control Technologies in the Food Industry), 179-196
CODEN: NSSSC9; ISSN: 1566-7693
PUBLISHER: IOS Press
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review. **Lactococcus lactis**, a common dietary supplement used for the manufacture of fermented foods, can be genetically engineered in such way that it secretes biol. active cytokines. When applied to the mucosa, the engineered strains can actively deliver these cytokines. By use of this principle - active in situ delivery of a therapeutic agent via de novo synthesis by genetically engineered bacteria - we developed a new therapeutic approach for inflammatory bowel **disease** (IBD), a group of chronic inflammatory **diseases** of the **intestinal** tract. 2 Animal models were used. Intragastric administration of L. **lactis** engineered to secrete murine interleukin-10 (mIL-10) produced a 50% reduction in **colitis** induced in mice by periodic addition of dextran sulfate sodium, as well as prevented the onset of **colitis** in IL-10-1- mice. This approach may provide a novel method for cost-effective and long-term management of IBD in humans.

REFERENCE COUNT: 102 THERE ARE 102 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L28 ANSWER 9 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2001:444123 BIOSIS
DOCUMENT NUMBER: PREV200100444123
TITLE: Treatment of murine **colitis** by **Lactococcus lactis** secreting IL-10.
AUTHOR(S): **Steidler, L. (1); Hans, W. (1);** Schotte, L. (1); Neirynck, S. (1); Obermeier, F.; Falk, W.; Fiers, W. (1); **Remaut, E. (1)**
CORPORATE SOURCE: (1) VIB-UG, Gent Belgium
SOURCE: International Journal of Antimicrobial Agents, (June, 2001) Vol. 17, No. Supplement 1, pp. S4-S5. print. Meeting Info.: 22nd International Congress of Chemotherapy Amsterdam, Netherlands June 30-July 03, 2001
ISSN: 0924-8579.
DOCUMENT TYPE: Conference
LANGUAGE: English
SUMMARY LANGUAGE: English

L28 ANSWER 10 OF 12 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2001010608 EMBASE
TITLE: A new approach to inflammatory bowel disease therapy.
AUTHOR: **Steidler L.; Hans W.;** Schotte L.; Neirynck S.; Obermeier F.; Falk W.; Fiers W.; **Remaut E.**
CORPORATE SOURCE: L. Steidler, University of Alberta, Faculty of Medicine and Dentistry, Division of Gastroenterology, Edmonton, Alta. T6G 2C2, Canada
SOURCE: Pediatric Research, (2001) 49/1 (2).

Refs: 2
ISSN: 0031-3998 CODEN: PEREBL
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 007 Pediatrics and Pediatric Surgery
026 Immunology, Serology and Transplantation
037 Drug Literature Index
048 Gastroenterology
LANGUAGE: English

L28 ANSWER 11 OF 12 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 6
ACCESSION NUMBER: 2000:278007 HCAPLUS
DOCUMENT NUMBER: 132:307260
TITLE: Use of a cytokine-producing **Lactococcus** strain to treat **colitis**
INVENTOR(S): **Steidler, Lothar; Remaut, Erik Rene**; Fiers, Walter
PATENT ASSIGNEE(S): Vlaams Interuniversitair Instituut voor Biotechnologie vzw, Belg.
SOURCE: PCT Int. Appl., 51 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000023471	A2	20000427	WO 1999-EP7800	19991006
WO 2000023471	A3	20000803		
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2343840	AA	20000427	CA 1999-2343840	19991006
AU 9963401	A1	20000508	AU 1999-63401	19991006
EP 1123314	A2	20010816	EP 1999-950738	19991006
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2002528392	T2	20020903	JP 2000-577196	19991006
US 2002019043	A1	20020214	US 2001-838718	20010419

PRIORITY APPLN. INFO.: EP 1998-203529 A 19981020
WO 1999-EP7800 W 19991006

AB The current invention relates to an administration strategy for the delivery at the intestinal mucosa of cytokines or cytokine antagonists, preferably of acid sensitive anti-inflammatory agents, such as IL10 and/or soluble TNF receptor via the oral route. The prefered feature according to the invention is the inoculation with a suspension of recombinant **Lactococcus lactis** cells, which had been engineered to produce the resp. proteins, e.g. interleukin 10, soluble TNF receptor, TNF antagonist, interleukin 12 antagonist, interferon γ antagonist, interleukin 1 antagonist and virus-coded cytokine analog EBV BCRF1. The recombinant

Lactococcus can be substituted with a Gram-pos. bacterial strain such as Bacillus subtilis, Streptococcus gordonii, Staphylococcus xylosus or **Lactobacillus**. The cytokine-producing Gram-pos. bacterial strain is especially useful for treating inflammatory bowel diseases such as chronic **colitis**, **Crohn**'s disease or **ulcerative colitis**.

L28 ANSWER 12 OF 12 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 7
ACCESSION NUMBER: 2000:622522 HCAPLUS
DOCUMENT NUMBER: 133:280359
TITLE: Treatment of murine **colitis** by **Lactococcus lactis** secreting interleukin-10
AUTHOR(S): **Steidler, Lothar**; **Hans, Wolfgang**; Schotte, Lieven; Neirynck, Sabine; Obermeier, Florian; Falk, Werner; Fiers, Walter; **Remaut, Erik**
CORPORATE SOURCE: Department of Molecular Biology, Ghent University, Ghent, 9000, Belg.
SOURCE: Science (Washington, D. C.) (2000), 289(5483), 1352-1355
CODEN: SCIEAS; ISSN: 0036-8075
PUBLISHER: American Association for the Advancement of Science
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The cytokine interleukin-10 (IL-10) has shown promise in clin. trials for treatment of inflammatory bowel disease (IBD). Using two mouse models, the authors show that the therapeutic dose of IL-10 can be reduced by localized delivery of a bacterium genetically engineered to secrete the cytokine. Intragastric administration of IL-10-secreting **Lactococcus lactis** caused a 50% reduction in **colitis** in mice treated with dextran sulfate sodium and prevented the onset of **colitis** in IL-10-/- mice. This approach may lead to better methods for cost-effective and long-term management of IBD in humans.
REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

FILE 'HOME' ENTERED AT 14:09:52 ON 09 OCT 2003